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Steroids, spinal cord and pain sensation

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Abstract

During the whole life, the nervous system is continuously submitted to the actions of different categories of hormones, including steroids. Therefore, the interactions between hormonal compounds and neural tissues are subjected to intense investigations. While a majority of studies focus on the brain, the spinal cord (SC) has received little attention, although this structure is also an important part of the central nervous system, controlling motor and sensory functions. To point out the importance of interactions between hormones and the SC in the regulation of neurobiological activities, we recapitulated and discussed herein various key data, revealing that the pivotal role played by the SC in nociception and pain modulation, directly depends on the SC ability to metabolize and synthesize steroidal molecules. The paper suggests that future investigations aiming to develop effective strategies against chronic pain, must integrate regulatory effects exerted by hormonal steroids on the SC activity, as well as the actions of endogenous neurosteroids locally synthesized in spinal neural networks.

Keywords: neurosteroid; nociception; pain; spinal cord.

Introduction

Because of their pleiotropic potential and diverse effects on the central (CNS) and peripheral (PNS) nervous systems, steroids have early been suspected to modulate pain sensation. Indeed, since 1927, Cashin and Moravek observed that intravenous injections of cholesterol were able to suppress pain sensation by exerting anesthetic effects in mammals [1]. Afterwards, Selye demonstrated that certain pregnane steroids, such as progesterone and deoxycorticosterone, can induce sedation and anesthesia in rats [2]. Together, these observations paved the way for the development of various synthetic analogs of pregnane steroids, which reduced pain through allosteric activation of $GABA_A$ receptors [3–7]. Nowadays, the therapeutic use of glucocorticosteroids and their analogs is considered as the most effective strategy against inflammatory pain, in spite of the occurrence of diverse side effects (for reviews, [8, 9]). Glucocorticosteroids reduce inflammatory pain by inducing anti-inflammatory actions on the damaged peripheral or central tissue which activates nociceptive mechanisms and generate pain sensation. The anti-inflammatory effects of glucocorticosteroids result from their ability to inhibit the expression of collagenase (the key enzyme involved in tissue degeneration during inflammatory mechanisms) and pro-inflammatory cytokines, or to stimulate the synthesis of lipocortin, which blocks the production of eicosanoids [10–13]. There is also clinical evidence supporting the use of glucocorticoids in the treatment of chronic neuropathic pain [14–17]. Experimental investigations in animals suggest that glucocorticoids may inhibit the initiation of neuropathic pain states, or attenuate this pain, but the mechanisms of action are unknown [14, 18–21]. It is usually thought that the anti-inflammatory actions of glucocorticosteroids may contribute to the inhibition of the neuroinflammatory component of neuropathic pain, but there is no specific evidence supporting this hypothesis. As an interesting finding revealed that the endoneurial expression of pro-inflammatory cytokines may have a role in the genesis of neuropathic pain, glucocorticosteroids may reduce this pain through the modulation of neuroimmune interactions [22]. In support of this idea, a recent study showed that the glucocorticoid triamcinolone, which reduced the neuropathic pain seen in the model of post-traumatic peripheral neuropathy, also decreased the number of endoneurial mast cells expressing (in the injured nerve) the pro-inflammatory cytokine tumor necrosis factor-alpha [23]. Altogether, the findings recapitulated above, strongly support the existence of key actions of endogenous and synthetic steroids in the modulation of inflammatory and neuropathic pain. The present paper aims to elucidate the specific contribution of steroid and SC interactions in the regulation of nociception and pain.

Background for a pivotal role of steroid and spinal cord interactions in the modulation of pain

The SC is a target of steroid hormones which are key factors accounting for the gender differences in pain and analgesia (for reviews, [24–28]). Variations in sex steroid levels, receptor expression and mechanisms of action in the nervous system, have been correlated with the development of chronic pain [24, 26]. Androgens, which are higher in males, exert analgesic effects in humans and experimental models, while estrogens were found to have both hyperalgesic and analgesic effects depending on the experimental conditions

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[28–31]. Moreover, it has recently been shown that progesterone prevents allodynia after SC hemisection-induced injury [32]. In addition to sex steroid-based dimorphism in pain sensation or in the risk of developing pathological pain syndromes, the relationship between opioids and steroid hormones in pain control has also been investigated by different research groups [33–36]. Investigations in humans and animals of the interactions between opioids and sex hormones in pain modulation, suggested that chronic opioid administration without testosterone supplementation may contribute to the perpetuation of chronic pain and to continued administration of unnecessarily high doses of narcotics [33, 34, 37, 38]. Verifications were made with hormonal supplementation in gonadectomized rats and the results indicated that testosterone plays a protective role in pain perception, while estrogens and progesterone mainly act on pain inhibition mechanisms [39, 40]. Studies focused on the rat SC indicated that sex steroids modulate antinociceptive responses to opioid drugs through the control of the expression of opioid kappa and delta receptors in spinal sensory neurons [41–43]. Progesterone-induced attenuation of pain behaviors in spinally-injured rats was correlated with increased opioid kappa receptor expression and upregulation of NMDA receptor subunits and protein kinase C gamma [32].

In addition to the effects of hormonal (adrenal and gonadal) steroids on the SC, the local production of endogenous steroids (neurosteroids) has been evidenced in the SC thanks to various investigations that revealed the presence and biological activity of several key steroid-synthesizing enzymes in the rat SC [44–48]. Among these enzymes, are cytochrome P450 side-chain-cleavage (P450scc), cytochrome P450c17 (P450c17), 3β-hydroxysteroid dehydrogenase (3β-HSD), 5α-reductase (5α-R) and 3α-hydroxysteroid oxidoreductase (3α-HSOR). P450scc catalyzes the conversion of cholesterol (CHOL) to pregnenolone (PREG), the first and rate-limiting step in the biosynthesis of all classes of steroid hormones. P450c17, also called 17α-hydroxylase/17,20 lyase, converts PREG successively into 17-hydroxy-PREG and dehydroepiandrosterone (DHEA). P450c17 is also responsible for the transformation of progesterone (PROG) into 17-hydroxy-PROG and androstenedione, successively. The enzyme 3β-HSD catalyzes the conversion of Δ^5-3β-hydroxysteroids (PREG, 17-hydroxy-PREG, DHEA) into Δ^4-3-ketosteroids (PROG, 17-hydroxy-PROG, androstenedione). 5α-R is responsible for the transformation of testosterone (T), PROG and deoxycorticosterone (DOC) into dihydrotestosterone (DHT), dihydroprogesterone (DHP) and dihydrodeoxycorticosterone (DHDOC), respectively. 3α-HSOR, also called 3α-hydroxysteroid dehydrogenase, converts in a reversible manner DHT, DHP and DHDOC into the respective neuroactive steroids 3α-androstanediol, allopregnanolone or tetrahydroprogesterone (3α,5α-THP) and tetrahydrodeoxycorticosterone (THDOC).

The first anatomical and cellular distribution of P450scc in the adult rat SC was provided by immunohistochemical studies, using two different antibodies against P450scc. One of these antisera was raised in rabbits against purified P450scc from bovine adrenocortical mitochondria [49–53].

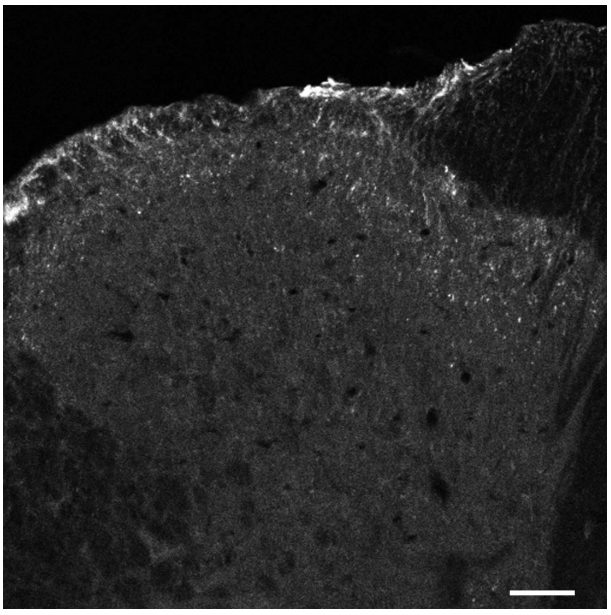

Figure 1 Transverse section through the lumbar SC showing P450scc-immunoreactive elements in the superficial layers of the SC dorsal horn (DH). Scale bar: 50 μm.

The other antiserum was generated in rabbits against the carboxy-terminal aminoacids 509–526 of rat P450scc [54]. The same anatomical and cellular distribution of P450scc-immunoreactivity was observed in the rat SC with both antisera. The highest density of P450scc-immunolabeling was found in superficial layers laminae I and II of the dorsal horn (DH) where sensory neurons are located (Figure 1). Double-labeling experiments revealed that most of the P450scc-positive fibers in the DH also expressed immunoreactivity for microtubule-associated protein-2, a specific marker for neuronal fibers [55–57]. Motoneurons of the ventral horn (VH) also expressed immunoreactivity for P450scc, suggesting a possible role of the enzyme or its steroid products in the control of motor activity [55, 58]. Moreover, P450scc-immunostaining was detected in ependymal glial cells bordering the central canal in the SC, an observation which suggests a possible release of neurosteroids in the cerebrospinal fluid and their involvement in volume transmission mechanisms in the CNS [55, 59].

Well validated biochemical experiments, which showed that homogenates from the adult rat SC are capable of converting CHOL into PREG, indicated that P450scc-like immunoreactivity detected in the spinal tissue corresponds to an active form of the enzyme [55, 60–64].

The demonstration of the presence and activity of P450c17 in the CNS has long remained controversial (for reviews, [65, 66]). Therefore, we combined molecular, immunohistochemical and neurochemical approaches for a solid investigation of P450c17 existence and biological activity in adult rodent SC. This multi-technique study allowed the first anatomical and cellular mapping of a biologically active form of P450c17 in the adult rat SC [67]. Significant amounts of P450c17 mRNA were detected in all regions of the SC, using

the real-time polymerase chain reaction approach after reverse transcription (RT-rtPCR). By taking advantage of the availability of an antibody against P450c17, we revealed the presence of a specific protein in total homogenates and microsomal fractions from the rat SC and testis. The P450c17 antiserum used in our studies was also efficient in previous investigations which localized the enzyme in Leydig cells [68, 69]. This antiserum also allowed the anatomical and cellular localization of P450c17 throughout the white and gray matters of the SC, using an immunohistochemical approach combined with confocal laser microscope analysis. P450c17-immunostaining was found in both neurons and glial cells. In the white matter, the enzyme was mainly detected in astrocytes, while in the gray matter, P450c17 was essentially found in neurons and oligodendrocytes [67]. The presence of P450c17 in the DH and VH suggested its potential involvement in the modulation of sensory or motor functions [58, 67, 70, 71]. Pulse-chase experiments, which revealed that SC slices converted [^{3}H]PREG into [^{3}H]DHEA, indicated that P450c17-like immunoreactivity detected in the adult rat SC, corresponds to an active form of the enzyme [67]. The occurrence of P450c17 enzymatic activity was further demonstrated with biochemical experiments using ketoconazole, a selective inhibitor of the enzyme [72, 73]. A significant decrease was observed in the conversion of [^{3}H]PREG to [^{3}H]DHEA by SC slices when the pulse-chase experiments were performed in the presence of ketoconazole, a result which unambiguously confirms the existence of P450c17 activity in the adult rat SC [67].

The first isolation of 3β-HSD mRNA in the SC was performed in rats using the RT-PCR approach [74]. However, the anatomical and cellular distribution of 3β-HSD mRNA in the SC was provided by Coirini et al. [75] utilizing an in situ hybridization technique. This study revealed that the DH laminae I-III exhibited the highest density of 3β-HSD mRNAs which were also detected in layer X around the central canal in the VH and in the lateral as well as ventral funiculi. At the cellular level, 3β-HSD mRNAs were found mainly in sensory neurons of the DH and in motoneurons of the VH throughout the cervical, thoracic, lumbar and sacral segments of the SC [75]. Moreover, evidence for the existence of 3β-HSD protein and enzymatic activity in the SC was provided by Western blot analysis and gas chromatography/mass spectrometry assays, which revealed that the concentrations of PREG and PROG were higher in the SC than in plasma [75]. Recent studies have also confirmed the presence and activity of 3β-HSD in the rat SC by using real-time polymerase chain reaction and pulse-chase experiments, combined with HPLC-Flo/one analysis of steroids newly-synthesized from a radioactive precursor in spinal tissue [76].

The expression of 5α-R in the brain, but not the SC, has extensively been studied [77–82]. It has been suggested that the isoenzyme 5α-R type 1 (5α-R1) essentially plays a catabolic and neuroprotective role, whereas the isoform 2 or 5α-R2 participates in sexual differentiation of the CNS. However, the neurophysiological significance of these two isoenzymes remains a matter of speculation [83–86]. The first demonstration of 5α-R gene expression in the SC was provided by a recent study, which revealed that, unlikely to what is observed in the brain, the quantity of 5α-R2 mRNAs extracted from the whole adult rat SC is higher than that of 5α-R1 [87]. This work also indicated that mRNAs encoding 5α-R2 are expressed by motoneurons of the VH, but did not provide any information about the presence or absence of the enzyme in the DH, where sensory networks are located [70, 71, 88]. Therefore, a detailed immunohistochemical study was performed to determine the regional and cellular distribution of 5α-R1 and 5α-R2 in the adult rat SC [89]. The study was possible thanks to the availability of highly specific antisera against 5α-R1 and 5α-R2 which were previously used with success to localize these enzymes in various steroidogenic tissues [90, 91]. Immunoreactivities for 5α-R1 and 5α-R2 were detected in the white matter of the SC from the cervical to sacral regions. However, the intensity of 5α-R1-immunostaining was low and cell bodies, as well as fibers containing this isoenzyme, were observed mainly in the white matter of the cervical and thoracic segments. The 5α-R2 immunofluorescence, which was moderate in the white matter, was intense in the DH and VH of the gray matter [89]. Double-labeling identification with specific markers for nerve cells revealed that the 5α-R1 immunostaining was mainly expressed in oligodendrocytes and astrocytes of the white matter, whereas 5α-R2-immunolabeling colocalized with neurons and glial cells in the gray and white matters [56, 57, 89, 92, 93]. The observation of a restricted localization of 5α-R1 to the SC white matter is in agreement with previous studies indicating that the type 1 isoform of 5α-R is the most relevant isoenzyme present in myelinated structures of the female and male rat brain [84, 94–96].

There are four human 3α-HSOR isozymes, but, to date, only one isoform has been cloned in rats [97–100]. The enzymatic activity and mRNA encoding 3α-HSOR have been detected in the brain, but the immunocytochemical mapping of the protein in the CNS has long remained unexplored [82, 101–103]. Taking advantage of the availability of a specific antiserum against the rat liver 3α-HSOR, we determined the anatomical and cellular distribution of the enzyme in the rodent SC (Figure 2). Relative titers, specificity and effectiveness of the 3α-HSOR antibody have been shown by previous biochemical and histochemical studies [97–99, 104, 105]. Intense immunoreactivity for 3α-HSOR was detected in SC white and gray matters. However, the highest density of 3α-HSOR-immunostaining was found in sensory areas of the SC [89]. Our study also revealed that 45% of 3α-HSOR-immunofluorescence was localized in oligodendrocytes, 35% in neurons and 20% in astrocytes. A comparative analysis of 5α-R1-, 5α-R2- and 3α-HSOR-positive elements in the SC, made it possible to observe three different but interesting situations: 1) cell bodies and fibers containing both 3α-HSOR and 5α-R were identified, 2) cells labeled only with the 5α-R1 or 5α-R2 antiserum were localized and 3) positive cell bodies expressing only 3α-HSOR-immunostaining were found [89]. Consequently, it appears that certain glial cells and neurons of the SC contain both 5α-R and 3α-HSOR enzymatic proteins, which could catalyze biochemical reductions required for the biosynthesis of

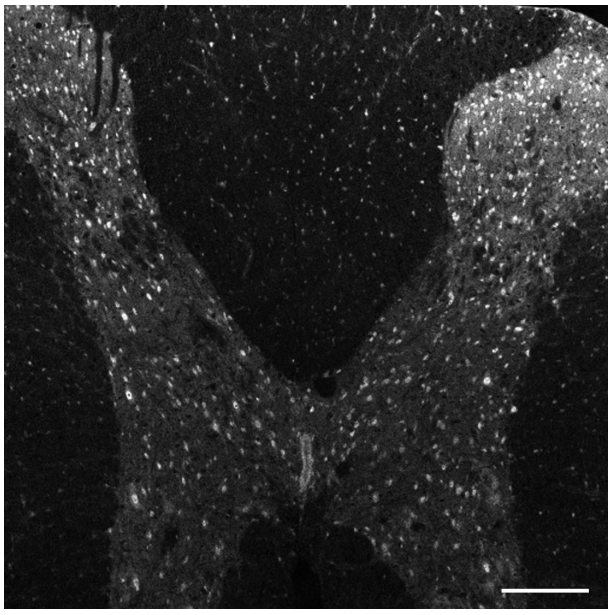

Figure 2 Transverse section through the thoracic SC showing 3α-HSOR-immunoreactive cells and fibers in the white and gray matters. 45% of 3α-HSOR-immunofluorescence was localized in oligodendrocytes, 35% in neurons and 20% in astrocytes. Scale bar: 200 μm.

3α,5α-reduced steroids, such as 3α-androstanediol, 3α,5α-THP and THDOC which control, through allosteric modulation of $GABA_A$ receptors, neurobiological mechanisms including stress, anxiety, analgesia, locomotion and lordosis [6, 106–109]. The production of neuroactive 3α,5α-reduced steroids may also involve collaboration among neurons, astrocytes and oligodendrocytes, which contain only one of the two enzymes, i.e., 3α-HSOR or 5α-R. This collaboration may be done within the context of the cross-talk between glial and neuronal elements in normal physiological or during pathological situations [110]. These suggestions could not rule out the possibility that 5α-R1 or 5α-R2 alone may convert, in the SC, PROG or T from peripheral sources, into DHP or DHT, respectively, that act via genomic receptors, the existence of which has been demonstrated in spinal tissues [111–114]. In a similar manner, 3α-HSOR alone may also convert, in the SC, peripheral DHP, DHT or DHDOC into 3α,5α-THP, 3α-androstanediol or THDOC, respectively, for the modulation of $GABA_A$ receptors [6, 109, 115, 116]. The fact that the rat spinal tissue homogenates are capable of converting [^{3}H]CHOL into various metabolites, including 3α,5α-THP, clearly indicates that 5α-R1, 5α-R2 and 3α-HSOR detected in the SC correspond to active forms of these enzymes [55, 89].

Role of spinal neurosteroids in pain regulation

Neurosteroids modulate $GABA_A$, NMDA and P2X receptors which are expressed in the SC and play a crucial role in the regulation of pain [45, 65, 71, 117]. However, the local synthesis of neurosteroids near their sites of actions in pain neural centers is a prerequisite to render credible the possible involvement of endogenous neurosteroids in pain modulation. Therefore, the demonstration that the spinal cord, which pivotally controls pain transmission [58, 71, 117], also contains the enzymatic machinery to locally synthesize neurosteroids (see above) was extremely important to show that neurosteroids are produced and released near their sites of actions in the spinal pain circuit. In addition, we observed that substance P, a major nociceptive neuropeptide secreted by primary afferents, inhibited in a dose-dependent manner allopregnanolone (3α,5α-THP) biosynthesis in the DH [118]. As the neurosteroid 3α,5α-THP is a potent allosteric stimulator of $GABA_A$ receptors, our observation suggested that substance P, by reducing 3α,5α-THP production, may indirectly decrease the spinal inhibitory tone and therefore facilitate noxious signal transmission.

To further investigate the possible role of neurosteroids endogenously produced in the SC in pain modulation, we performed a multidisciplinary study using the rat experimental model of neuropathic pain generated by sciatic nerve ligatures [119]. Molecular and biochemical investigations (quantitative real time polymerase chain reaction after reverse transcription, Western blot, radioimmunoassay, pulse-chase experiments, high performance liquid chromatography and continuous flow scintillation detection) revealed an up-regulation of enzymatic pathways (P450scc and 3α-HSOR) leading to 3α,5α-THP biosynthesis in the SC [89, 120, 121]. In contrast, the biosynthetic pathway (P450c17) producing DHEA was down-regulated in the neuropathic rat SC [67, 122]. Behavioral studies using the plantar test (thermal nociceptive threshold) and the von Frey filament test (mechanical nociceptive threshold) showed that intrathecal administration of 3α,5α-THP in the lumbar SC induced analgesia in neuropathic-pain rats, by suppressing the thermal hyperalgesia and mechanical allodynia characterizing these animals. Unlike 3α,5α-THP, intrathecal injection of Provera (3α-HSOR inhibitor) potentiated both thermal hyperalgesia and mechanical allodynia in neuropathic rats [121]. Moreover, in vivo knockdown of 3α-HSOR expression in healthy rat lumbar dorsal root ganglia using 6-carboxyfluorescein-3α-HSOR-siRNA, exacerbated thermal and mechanical pain perception [123].

Acute DHEA treatment exerted a rapid pro-nociceptive and a delayed anti-nociceptive action. Inhibition of DHEA biosynthesis in the DH by intrathecally administered ketoconazole (a P450c17 inhibitor) induced analgesia in neuropathic rats. Chronic treatment of DHEA increased and maintained elevated basal pain thresholds in neuropathic and control rats, suggesting that androgenic metabolites, generated from daily injected DHEA, exerted analgesic effects while DHEA itself (before being metabolized) induced a rapid pro-nociceptive action [122].

In agreement with our findings showing spinal neurosteroid involvement in pain modulation, various other investigations using synthetic analogs of 3α,5α-THP also revealed antinociceptive properties of neurosteroids in humans and

animals [124–128]. Furthermore, it has clearly been demonstrated that 5α-reduced neurosteroids induce a potent peripheral analgesia which is mediated by both T-type calcium and $GABA_A$ channels [129].

Conclusion

Pain, the first reason for medical consultation in many diseases, is a complex process involving several molecular, cellular and integrated mechanisms as well as psychosocial parameters. The data reviewed herein demonstrate that the interactions between steroids (hormonal steroids and spinal neurosteroids) crucially regulate diverse processes determining the sensitivity to pain. The paper suggests that the investigations aiming to develop effective strategies against chronic pain may particularly integrate the pivotal role played by spinal endogenous neurosteroids in the control of nociceptive transmission.

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